



Accessibility of ureolytic strains of bacteria for production of ureases organic base bio fertilizer

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General Note



Article is recommended to print as color version in recycled paper. *Save Trees, Save Nature.*

ABSTRACT

The diversity of tiny plants /microorganisms and their associates are governed by wide array of intricate natural factor like those of temperature, relative humidity, light intensity etc respectively. Their presence plays a significant role in the growth of plants, animals/ microorganism in their unique endemic consortium within a given habitat. Further investigations were cautiously made on the quality of top surface soils for both control farm land and contaminated land fill, thus they are found to be quite significantly different in their soil texture, permeability, colour etc respectively. Isolation of bacteria isolates, Characterization, and biochemical identification from tannery sludge was carried out in due course. The bacteria isolates that were significantly and namely identified are as follows; (*Proteus vulgaricus*, *Klebsiella* species, *Pseudomonas aeruginosa*, *Bacillus* Sp) etc respectively. All the isolates were

found to be Gram +ve,, motile and Klebsiella Sp were found to be capsule staining, and ureae activities, catalase test, oxidase test were all positives. Effect of metal ion concentration of ureolytic strain of bacteria were duly accentuated as well, the results revealed HgCl and CrSO₄ concentration at 2% most organisms growth were –Ve or death while other remain +ve or viable. Tolerance level of ureolytic bacteria growth regarding to pH and temperature results were also computed and found that at pH3-6 none are viable, but at pH7 there was an appreciable and significant growth. Similarly to the temperature at 24°C there was no trace of microbial growth, at 27°C-33°C there was an appreciable and maximum growth with immediate alacrity. Nutritional values of the carrier base or organic waste like those of bean pod waste, rice bran waste, banana peel waste etc respectively, were observed and there was significant nutritional values for both plant and microorganisms growth without qualm. Proximate analysis of the nutritional values of both organic base urease bio-fertilizer/ bio-seed bio-fertilizer like those of Ash, moisture crude proteins etc respectively were cautiously accentuated in situ. The study regarding to the results or findings were checkmated to have mostly found that the organic bio-fertilizer are far much higher than the urea chemical recipes. In view of this current investigation, these properties suggest that ureolytic strain of bacteria isolated in this study could find potential application for development of the sustainable agriculture as to be a good candidate of bio-fertilizer which help in soil fertilization without applying chemical fertilizers.

Key word: Ureolytic strains of bacteria, Bio-fertilizer production, Multiple carrier organic base waste, Macro element/ microelement

1. INTRODUCTION

One of the major concerns in today's world is the pollution and contamination of environment. The contamination does not restrain only within soil, water and air rather it spreads within the things those are living in the environment by chain. The use of chemical fertilizers has tremendous impact on environmental pollution. Fertilizer is commonly use in agricultural activities, lawn and garden maintenance. The main constituent of fertilizer is usually nitrogen. As matter of truth the crops are not enough efficient to use it and consequently a greater proportion of the chemicals is lost and/or some undesirable toxic substances are released in the soil, water and gaseous systems (Akbari, *et al*, 2011). Inappropriate use of chemical fertilizer aggravates the problem resulting in deterioration of soil, water and food quality and in gaseous imbalance in the atmosphere. Many studies on contamination of soil, groundwater, surface water and air by Nitrogen fertilizer and a few researches on impact of Nitrogen fertilizer on human health are conducted by the various researchers. All these cases the researchers mentioned that the cause of contamination is heavily fertilized above the recommended level. The studies show that long term application and excessive deposition of nitrogen fertilizer in soil affect on biological and biochemical activities of the soil and hence reduce the fertility (Brown, 2012) Soil microbes and soluble Nitrogen fraction is reduced due to the use of nitrogen fertilizer and water contamination risk is increased (Karakurt and Aslantas, 2010). The functional diversity of soil Nitrogenous Fertilizer (N) has become the key input in food production worldwide. Cereals (rice, wheat, maize, millet, etc.) account for more than half of the total N fertilizer consumption in the world, and approximately 50 – 70% more cereal grain will be required by 2050 to feed over 9 billion world population (Rivera-Cruz *et al.*, 2008). This will further increase demand for N fertilizer at greater magnitude unless the N fertilizer recovery efficiency in cereals is improved through better production technologies. Increasing cropping intensity with modern rice varieties in irrigated rice farming system in Ghana, has enhanced nutrient mining from the soil because nutrient removal has exceeded annual replacement, even if the national recommended fertilizer doses are applied. Long-term studies done by (Abou El-Yazied and Sellim, 2007) attributed the reduced productivity of the rice system to declining soil organic matter (SOM), decreased soil fertility, and occurrence of nutrient imbalances. The cultivation of rice under continuously flooded conditions for several years, hinder the decomposition of organic matter (Mosa *et al.*, 2016). Hence less nitrogen and other nutrients are released to the plants. Therefore, to maintain the required yield, that larger amount of manures and fertilizers must be added. Adding organic matter to the paddy soil may increase the soil fertility. And as far as plant nutrition is concern, organic matter can be critical in the supply of nitrogen, sulphur and micronutrients to the rice plant. These are released in plant available form as the organic matter mineralizes (Yang *et al.* (2014). The potentiality of organic waste in crop production systems is very limited due to its slow release of plant nutrient to synchronize with crop demand a particular stage of growth. Only one fifth to half of the nutrient supplied from manure was recovered in the first year and the rest released gradually to succeeding crops, thus showing residual effect of OM application (Grzyb *et al.* 2015). However, (Trias *et al.*, 2008) asserted that residual effect of N- and P-based manure and compost application on corn yield and N uptake can last at least one growing season, while (Asharf and Khan, 2010) stated that long term cultivation without the application of organic fertilizers decreased organic carbon and total N contents of the soil. Moreover, the incorporation of compost into the soil increases the soil organic carbon content (Mazzila *et al.*, 2009).

In view of the current investigation was therefore to convert inorganic urea using microbial enzymes (Urease) for urease organic bio fertilizer on grain yield nitrogen use efficiency of numerous irrigated crops like those of Rice, maize, millet as well as Yam tubers etc respectively.

Statement of problem

In recent years, it is being observed that excessive exposure to chemical fertilizers like those of urea as well as N.P.K which does not only deteriorate soil health but also create several environmental impacts as global threat to humanity. Consequently the threats to mankind have always been observed to the kind of Chemical fertilizer bi- product that men have been acquainted for ingestion as food on daily bases. Thus the deadly disease like those of diabetes and High blood pressure are the major prerequisites that curtail the life shelf or span of mankind of not less than 20 to 80 years and above are victim of the dreaded syndrome statistically. For every average Nigeria both young and elderly by medical statistic 25 years and above have perpetually acquired High blood pressure as well as pill due to indiscriminate in ingestion of chemical fertilizer by-products.

Objectives

To evaluate effective used of urease base organic bio-fertilizer on the growth response of cereal crops

Specific objective

- 1.To select the ureolytic bacteria strains that are responsible or suitable for the production of Urease Enzyme
- 2.To evaluate the effect of the new improve organic base bio-fertilizer growth and development of cereal crops
- 3.To determine the optimum dose of Urease Base organic bio-fertilizer food crops growth response and recommend for the growers.

Significance of the study

The study has been view to have the following important points. Server as general guide line for farmers or development agency; Providing an excellent starting point for further investigation service as mean of increasing the yield of cereal crops food.

2. LITERATURE REVIEW

Role of urea in plant nutrient

Nitrogen is the most abundant nutrient in plant; it constitutes 2-4% of plant dry matter. Nitrogen is the key nutrient input for achieving higher yields of cereal crops. Most cereal crops are quite sensitive to insufficient nitrogen and response to nitrogen fertilizer. Nitrogen plays a central role in plant biochemistry (Alam *et al.*, 2007) indicated that the most important role of nitrogen in plant is the presence in the structure of the protein and nucleic acid which are the most important building block of life from which the living material or protoplasm of every cell is made. In addition, nitrogen is also found in chlorophyll the green coloring matter of plants. Since Nitrogen is part of so many without added N is slow (Maqsood *et al.*, 1995). Nitrogen is an indispensable element constituent of numerous amino acid, proteins, nucleic acid and hormones (Mengel and Kirkby 1987). It is involve in all major processes of plants growth and development and yield formation. Beside a good supply of nitrogen to the plant stimulates root growth, development as well as uptake of other nutrients (Olsen and Cole, 1954). Nitrogen is responsible for the dark green color of the stem and leaves, vigorous vegetable growth branching /tillering leaf production, size enlargement and yield formation. Adequate supply of Nitrogen is associated with high photosynthetic activity. According to (Mohammed *et al.*, 2010), Nitrogen is well property in conjunction with other. Soil fertility inputs can speed up the maturity of crops such as maize and small cereals. The supply of Nitrogen is related to carbohydrate utilization. When N supplied is insufficient, carbohydrate will be deposited in vegetative cells causing them to be thickening where as under adequate supply and favorable condition for growth. Proteins are formed from manufactured carbohydrate resulting in more protoplasm (Havlin *et al.*, 1999).

A low supply of Nitrogen has a profound influence on crop growth and may lead to a great loss in grain yield (Ottman, 2009). Nitrogen deficiency in plants results in a marked reduction in growth rate. A deficiency of N limits cell division and expansion of chloroplast development, chlorophyll concentration and enzyme activity. Nitrogen deficient plants have a short and spindly appearance. Tillering is poor and leaf area is small. As nitrogen is a constituent of chlorophyll, its deficiency appears as a yellowing or chlorosis of the leaves. This yellowness usually appears first on the lower leaves while upper leaves remain green as they receive some Nitrogen from older leaves remain green. The effect of Nitrogen toxicity is less evident than those of its deficiency. They includes prolong growing vegetable period and delayed crop maturity. High ammonium in solution can be toxic plants growth

particularly where the solution is alkaline. The toxicity results from ammonia (NH_3) which is able to diffuse through plant membrane and interfere with plant metabolism.

Soil conditions and urea uptake

Nitrogen is one of the most widely distributed element in nature and the atmosphere is the main reservoir. The soil accounts for only a minute fraction of lithosphere N and the soil Nitrogen only but a very small proportion is directly available to plants in the form of NO_3^- and NH_4^+ ions. Nitrogen is a very mobile element circulating between the atmosphere, the soil and the living organisms (Mingle and Kirkby, 1987). Inorganic Nitrogen exist in the form of NH_4^+ , NO_3^- , NO_2^- , N_2O and element $;\text{(N}_2\text{)}$. The organic forms include proteins, amino-acid, amino sugar and other like those of NO and NO_2 which are produced from aerobic decomposition of organic matter or addition of fertilizers to the soil and are most important in plant nutrition. Gaseous N_2 , N_2O and NO are form of Nitrogen lost through denitrification (Tigre *et al.*, 2014).

Nitrogen is a unique plant nutrient, since plants absorb both NH_4^+ and NO_3^- . The ratio of ammonium to nitrate in the soil depends on the presence of satisfactory conditions for nitrification which is inhibited by low soil pH and anaerobic conditions. The type and age of plant, the environment and other factor determine preference of plants either to NH_4^+ or NO_3^- ion. For most crops, the N form (NH_4^+ or NO_3^-) is of minor importance although some plants appear to have a specific preference for one or the other crops would prefer (NH_4^+) as it is directly usable for protein synthesis where as NO_3^- most first be reduce to NH_4^+ which requires energy. Crops can utilized. The NH_4^+ or NO_3^- form of N. slightly higher seed set has obtained with the NH_4^+ form.

Arable crops mainly take up NO_3^- even NH_4^+ fertilizer are in the soil. However, plant growth is improved when the plants are nourished with both NH_4^+ and NO_3^- compared to either NH_4^+ or NO_3^- alone grain yield increase from 7- 47% with NH_4^+ and NO_3^- compare to yields with NO_3^- alone which was related to increase number of tillers and kernels per plant (Abdullahi *et al.*, 2012).

In normal cropped soils where ammonium is added through fertilizer or release from organic matter or crop residue by mineralization. It is usually nitrified rapidly to Nitrate Nitrogen added in the Amide forms as in urea is first hydrolyzed to ammonium with the help of urease enzyme. It can then be absorbed by roots as such or converted to nitrate and then absorbed.

Effect of urea fertilizer on the growth and yield components of cash crops

According to Burger and Berge, Nitrogen is a major requirement for high yields of cash crops and nitrogen fertilizations are often essential on soils of low organic matter content or when re-cropping after non- legumes. Crop response to Nitrogen fertilizer is influenced by factors such as nitrogen fertilizer management. Soil type crop sequence and supply of residual and mineralized nitrogen when plants are deficient in Nitrogen, they become stunted and yellow in appearance. Nitrogen deficiency in cereals results in restricted root growth, poor tillering, thinner and smaller stems, premature ripening of grains and low number of ears per unit area and low number of grains per ear (Mengel and Kirkby, 1987). The grains are small but often relatively high in protein. Content due to a decrease in the input of carbohydrate into grains during the later stages of the grain filling stage (Mengel and Kirkby, 1987).

Important of nitrogen to cereal crops growth and development

Nitrogen is one is one of the major plant nutrient were satisfactory level of grain and foliage production on Vertis soil depend on its adequate supply. Although nitrogen requirement of crop met through addition of nitrogen fertilizer, it is an expensive input and those reflect its consumption in both Nigeria high and low land. Nitrogen plays vital role in all living tissue of plant. No other elements have such an effect on promoting vigorous plant growth. Abundant of proteins tend to increase the size of the leaves accordingly to bring about an increase in carbohydrate, synthesis. Nitrogen plays a vital role in increasing the yield of crops. The application of prop per amount of nitrogen is a key to obtain better crop of cereal crops. High nitrogen supply favors the conversion of carbohydrate into proteins which in turn promotes the formation of protoplast. Split N- application has little effect on yield but could result deceased logging and spike population while grain weight is increase (Mengel and Kirkby, 1987).

2. MATERIALS AND METHODS COLLECTION

Collection of soil and sludge sample

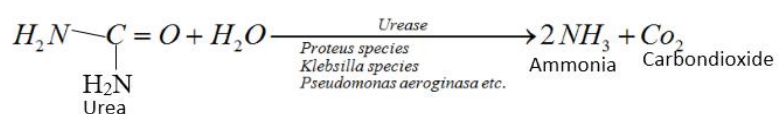
The study was conducted by randomly collection of soil samples from NARICT Control farm Land, and Yankusa land fill. Collection of sludge sample from Mario Jose tannery Kano and other various parameters like those of soil, structure, texture, permeability, pH and Temperature were instantly and duly taken or recorded and kept in the Laboratory for futuristic analyses. The average rainfall recorded in those areas 120 mm. The mean annual temperature varies between 33°C and 34°C . The species diversity were determine by employing quadrant method in an area of 1x1m randomly selected at different points, maintaining a minimum

distance of about 50m. The study was conducted over a period of 3-4years as the occurrence species was seasonal. Other parameters viz; relative humidity, temperature, light intensity were recorded using hygrometer, thermometer and lux meter respectively.

Descriptions of the modified experiment

Principle/ Theory

Urea base organic fertilizer (principle): urea is a major organic waste product of protein digestion in most vertebrates and is excreted in the urine. Some microorganisms have the ability to produce enzyme urease is hydrolytic enzyme which attacks the carbon and nitrogen bond Amide compound e.g. urea with the liberation of ammonia as shown below



Preparation of modified urease medium

Urea medium with the following constituent g/l are as follows;

Meat extract/chocolate blood paste- 1000ml

NaCl- 5g

KH₂PO₄- 4g

Natural Glucose (Dorowa) - 80g

Molasses 40g

Urea- 200g

pH 6-7.2

Distilled water 4000ml

The medium was dissolved by heating and adjusting the pH to 6.8 and autoclave at 121°C for 15minutes and cool to 50°C

Preparation of urease base organic fertilizer



Plate 1 posted different organic waste as carrier base

1000ml of semi-digested blood extract/ an appreciable amount of NaCl was poured into 1000ml beaker, in a short while the solution was later turn into 6000ml pot. 2000ml of blood paste was meticulously introduce into the net resultant solution in the pot and 1000ml of distilled water which was then allowed to boil for 30 -40 minutes. After the solution was boiled for required desire then semi liquid 250g of Dorowa in addition to 250g of sugar cane molasses plus 250g of soya bean meal were dissolved in 3000ml bowl containing 1000ml of water to form a paste. The paste solution was later poured into the same pot for further boiling to obtain desire requirement. The semi liquid boiling solution was further added concomitantly with 250g of Dorowa, 250g of sugarcane molasses, 250g of soya bean meal respectively into the same boiling pot for while until a semi solid paste was obtained once again.

By and large 500g of rice husk bran, 500 dry blood powder meal, sawdust respectively were concomitantly added to the semi solid paste and there upon allowed to maintain it solidity before pour spray on a sterile tray. The semi solid urease organic fertilizer was then sundry or oven dried at 37- 40°C(semi fermented urease organic fertilizer) along side with the bacteria until its dried up to form a compost cake as seen in plate 1 and 2.



Plate 2 posted composites organic base cake bio-fertilizer

Extraction of enzyme urease from bacteria

High speed refrigerated centrifuge was improvised and two days culture bacterial culture was poured into centrifuge tubes which was then allowed to spin for 20 minutes at 5000rpm after which the supernatant was decanted to obtain crude enzymes as seen plate 3.



Plate 3 posted purified ureolytic bacterial strain

Bacterial consistency/viability in urease organic base bio- fertilizer

Bacteria consistency is a method employ in a bio-seed coated with organic urease bio fertilizer so as to checkmate the viability of urease organisms whether they are still alive for the production of another fresh urease base organic bio-fertilizer even when the organisms were not available for isolation in inoculating chamber as shown in plate 4.



Plate 4 posted ureolytic strain of bacteria viability

Chocolate blood paste preparation

Sterile Blood sample was specifically processed for sole source nitrogen which is best known as biocatalyst for microbial growth during urease base organic bio-fertilizer production. The sterile raw blood was conveyed right from the slaughter house to NARICT laboratory which was scientifically processed by boiling half way to form a semi digested protein. There upon the solution was filtered to obtain blood extract. An appreciable amount of salt was added to produce peptone extract; the solid blood residue was finally cut into choppy small substances using Knife and was finally blended using blender to obtain blood paste for nitrogen source in readiness for urease base organisms growth as picture in plate, 5 and 6.



Plate 5 posted fresh cow blood from slaughter house



Plate 6 posted process blood paste chocolate

Isolation of urease base microorganisms from tannery effluent waste

Thirty ml of tannery sludge waste was dissolved in fifty ml distilled water in 250ml beaker and left for 3 days aseptically. Ten ml sludge waste was transferred in 90ml of sterile distilled water and aseptically serial dilution was performed. Ten ml of the sludge waste was pipetted into 90 ml water blank number 2 to make up 1:100 (10^{-2}) and another 10ml of Effluent waste was pipette into 90ml water blank number 3 to make up 1:1000 (10^{-3}). Further dilutions were repeatedly carried out for 10^{-4} to 10^{-7} respectively. Aliquots 0.1ml was aseptically transferred into freshly prepared potato dextrose agar medium. Sterile glass spreader was used to aseptically spread the sample on the surface of agar medium. The plates were then incubated at ambient temperature ($27-32^{\circ}\text{C}$) for 3-4 days. Emerging bacterial colonies were picked and aseptically sub cultural into freshly prepared Nutrient agar and Incubated to obtain a pure isolates. These pure isolates were then kept on Nutrient Agar slants at 4°C for further stored (Adawiah, 2008). Bacteria were also isolated from sludge waste and reference water sample by the sample the same procedure.

Biochemical test for urease base microorganisms

Identification and biochemical characterization of bacterial strains isolated colonies after purification were initially gram stained. By using bergey's manual of determination of Bacteriology (9th edition), the isolates were biochemically characterized and identified as follows. Catalase test, oxidase Test, spore staining, starch hydrolysis, citrate utilization test, methyl red Voges Proskauer test (MR-VP),

nitrate Reduction test gelatin liquefaction test, triple sugar iron test, lactose/glucose fermentation, indole production test, Urease test, motility test etc (Pervin *et al*, 2017).

Identification of urease base microorganisms

The bacteria that were identified for urease organic Base bio-fertilizer are as follows: *Bacillus* species, *Pseudomonas* species, *Proteus* Species, *Klebsiella* species plate

Determination of the physicochemical properties of rhizobium base organic biofertilizer

pH

Ten grams of the samples was taken and added to twenty five ml of distilled water. The mixture was shaken intermittently for 30 minutes. The pH was then determined by using the pH meter in standard bulb solution (Nag, 2007).

Organic Carbon

The method used was by (Fawole, and Oso, 2004). Two and half gram of dried, sample was taken into a pre-weight crucible and ignited over a Bunsen burner to a bright red heat, stirred occasionally with a wire loop. The sample was heated for 15 minutes. There upon it was then allowed to cool in a desiccators and the weight of buffing dust was taken. The organic carbon content was calculated as followed.

$$\text{Percentage organic matter} = \frac{\text{loss in weight}}{\text{Weight of sample}} \times 100$$

$$\text{Percentage organic carbon} = \frac{\text{organic matter}}{1.72}$$

Determination of ash content

A porcelain crucible was dried in an oven at 100°C for 10 minutes, cooled in a desiccator and weighed (W_1). Two grams of the sample (buffing dust) was placed into the weighed porcelain crucible and re-weighed (W_2). It was then transferred into a furnace, which was set at 550°C. The sample was then left in the furnace for 6 hours to ensure proper aching. The crucible containing the ash was then removed cooled in the desiccators and weighted (W_3). The percentage ash content was calculated as:

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where,

W_3 = weight of crucible and the content after cooling in the dessicator

W_2 = weight of content and crucible before heating

W_1 = weight of the crucible (AOAC, 1984)

Determination of crude fiber

Two grams of the finely sample was weighed out into a round' bottom flask and mixed with 100 ml of 0.25M H_2SO_4 . The solution was thoroughly mixed and boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed severally with hot water until it was acid free. This was later transferred into a flask and 100 ml of hot 0.31 M NaOH solution was added and the mixture boiled again under reflux for 30 minutes and then quickly filtered under suction. The soluble residue was washed with boiling water until it was base free and later dried to a constant weight in the oven at 100°C and finally cooled in a dessicator and weighed (C_1)

The weighed sample (C_2) was then incinerated in a Gallemkamp (80) muffle furnace at 550°C

$$\% \text{ crude fibre} = \frac{C_1 - C_2}{\text{Wt of sample}} \times 100$$

Wt of sample (AOAC, 1984)

Temperature

Ten grams of the sample was taken and added to 25ml of distilled water and the mixture was shaken thoroughly for 20 minutes. The temperature was determined using the thermometer in solution (Aneja, 2007).

Organic matter

Two and a half grams of dried, sample was taken into a pre-weighed crucible and ignited over a Bunsen burner to a bright red heat, stirring occasionally with a wire loop. The sample was heated for 15 minutes. Then it was allowed to cool in a desiccator and the weight of the soil was taken. The organic carbon content was calculated as follows:

$$\% \text{ Organic matter} = \frac{\text{loss in weight}}{\text{Weight of Sample}} \times 100$$

Fawole and Oso, (2004).

Determination of total carbohydrate content

The total carbohydrate content of the sample was obtained as described by Moronkola *et al.*, (2011), where the results from fat, protein, moisture and ash content analyses were sum-up and the carbohydrate content was calculated as follows: 100 - (% moisture + % protein + % fat + % ash).

Total nitrogen (N)

One and a half gram of crushed dried samples was pour into 300ml Kjeldal flask along with 25ml of concentration. H_2SO_4 and 3g mixed catalyst. The sample was digested using Kjeldahl digestion apparatus until a clear green or whitish color was obtained. The digested solution was then diluted to 100ml with distilled water. Distillation was done adding 20ml of diluted digest into 500ml Kjeldahl flask containing anti – bumping chips and 40ml of 40% NaOH was slowly added by the side of the flask. A conical flask (250ml) containing a mixture of 50ml 2% boric acid and 4 drops of mixed indicator (Cresol/bromothymol) was used to trap the liberated ammonia. The distillate was then titrated with 0.1m HCL. The total nitrogen content was then calculated using

$$\% \text{N}_2 = \frac{14 \times M \times V + x \times V}{\text{weight of sample (mg)} \times V_S} \times 100$$

Where M = Actual molarity of acid
 V = Titre volume of HCL used
 V+ = Aliquot volume distilled
 (Onyeika and Osieji 2003).

Phosphorus (P)

Fifty grams of the dried crushed Rhizobium organic base Biofertilizer was suspended and filtered through a nylon cloth into a glass beaker. Twenty five ml of the filtrate was heated for 25 minutes with HNO_3/HCL in a ratio of 3:1 (digestion). The mixture was dilute was diluted to the 100ml mark with distilled water. Fifteen ml of the diluted solution was then pipette into a cuvette and 1ml of the phosphate reagent was added to it and the reading taken using the phosphate meter (Nag, 2007).

Potassium (K)

To determine the potassium content of the samples fifty grams of the dried Urease organic base Bio-fertilizer was suspended in 50ml of distilled water and filtered using nylon cloth. The filtrate (25 ml) was mixed with $\text{HNO}_3/\text{HClO}_4$ (ratio 2:1). The beaker containing the mixture was then placed on a hot plate and boiled until the solution became clear. This was then filtered using what man filter paper No.1 in a volumetric flask and the volume of the filtrate was made up to 100 ml by the addition of deionized water digested sample was stored in a sterile polyethylene bottle at room temperature for further analysis of the metal using atomic absorption spectrophotometer.

Calculation

$$\% \text{ Potassium} = 2 \times 0.005$$

Where R = Potassium Concentration (ppm) in the aliquot (Nag, 2007).

Manganese determination

Hundred ml of urease organic base bio-fertilizer sample was processed from hundred gram of the sample. After which 5ml special reagent was added. There upon it was concentrated up to 90ml through boiling. 1g of $(\text{NH}_4)_2 \text{S}_2 \text{O}_8$ was added and was later boiled for 1 minute. After which the removal of the heated solution from the heat source was later allowed to stand for 1 minute. The solution was then diluted to 100ml with distilled water. Finally preparation of standard containing 100- 1500 μg manganese was treated using treated using various amounts of standard manganese solution in like manner.

Calculation

Manganese $\text{mg/l} = \mu\text{g}(\text{in } 100\text{ml final volume/ ml sample})$ (Nag, 2007)

Magnesium determination

Fifty ml of urease organic base bio-fertilizer was processed from fifty 50 g of sample. Concomitantly, a few ml of concentrated hydrochloric acid (HCl) was added to the solution. There with, 10ml of 10% solution of ammonium chloride was added to make alkaline with concentrated ammonium hydroxide and was gently boiled for a few minute. Constant stirring of the solution to warmness was made after which 10 ml of saturated solution of ammonium oxalate was added too. In the process of time the solution was allowed to stand for 30minutes. Filtration was followed using quantitative filter paper on a funnel. Soon after, a concentration of ammonium hydroxide to filtrate was made up to equality of one-ninth of its volume. Furthermore 10ml of 10% solution of diammonium hydrogen phosphate was added to the solution for at least 3hours and then finally filtered. Thereupon the folder paper was transferred a long side with magnesium precipitate in a weigh crucible. Soon after the precipitate was thoroughly dried in a hot air oven and therewith the crucible was ignited along side with magnesium precipitate for 3 minutes at 1000°C . Finally the crucible along with precipitate was maintained in a dessicator/ crucible until constant weight was duly obtained.

Calculation

Magnesium $\text{mg/litre} = \text{mg Mg } 2 \text{ P}_2\text{O}_7 \times 218.4/\text{ml of sample}$ (Nag, 2007).

3. RESULTS AND DISCUSSIONS

The diversity of these tiny non-veg plants and their associates are governed by wide array of intricate natural factors within the population level (Satyanarayana *et al.*, 2005). The habitat preference which in turn is due to the conducive micro climatic conditions was the key factor that sustains the unique endermic consortium. The spores and seeds germinate within a fortnight after a downpour and the diversity recorded was maximum during the period October. Properties of the soils at their consortium of both control land fill and contaminated landfill were taken in to recognizance in situ as shown in table 2 the control farm land (NARICT) has really shown a remarkable and better structure, texture, composition etc for soil that could be able to sustain quite a truck load of microorganisms, plants and humans with regard to the said contaminated landfill of Yankusa Kano. Thus this is basically made of up obnoxious and nuisance industrial discharge from tannery waste effluent. The emissions of effluent containing toxic chemical recipe have drastically rendered the virgin soil unproductive and devastated to plants and animals (Diaz, 2008).

Soil is a potent system of terrestrial system, and direct discharge of industrial tannery effluent especially that without treatment may have profound influence on physiochemical and biological properties of soil fertility (Satyanarayana *et al.*, 2005). This study is further view with respect to NARICT farm land areas are quite a better soils for sustainable microbial activities in given habitat in table 2. The consolidated and synergistically relationship played between the plant root and microorganisms have always been totally and ad equally a well come development for every year bumper harvest of crop to farmers, thus irrespective of the application of Chemical fertilizer recipes. The mineralized elements that are readily made soluble for plant growth are always available, and thereby creating the variable existence of the array of species' of plant growth; which is attributed to the uncontaminated rain water source (Bodelier and Dedysh, 2015). Apart from the polluted discharge from tanneries, there were also some human activities that could warrant the deterioration of the said consortium of microbial activities in a given habitat to negativisms. For instant the discharge of chemical recipes like those of Chromium ion(Cr^{3+}), chemical pesticides etc could ultimately degraded the ecosystem to nothingness permanently (Diaz, 2008).

The bacterial cultures were isolated from sludge tannery sample collected from Mario Jose Tannery industry Kano state. The bacterial samples were identified by morphological and biochemical tests for presence of phosphate solubilizing as well as ureolytic bacteria. There with all the isolates were subjected to various testes for confirming their identity in table 3. All the check isolated and standard strains formed completely white, round, smooth and shiny colonies. During microscopic observation all the isolates were

found to be gram positive and rods shape. Presence of endo-pores was confirmed by endospore staining but non-sporeformers. Virtually all the bacterial isolates were ultimately motile when observed under light microscopy in table 3.

Bacterial isolates namely; *Bacillus* sp, *Pseudomonas* sp, *Proteus mirabilis*, *Klebsiella* sp, and *Enterobacter* were characterized under standard methods as shown in table 4. Plate 7, 8, 9, 10 and 11.

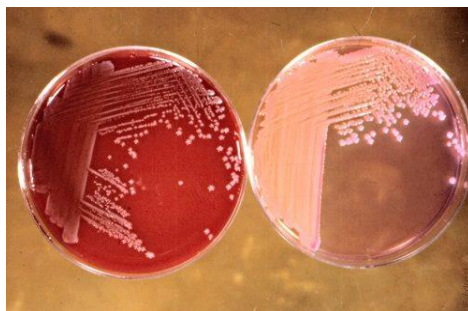


Plate 7 posted pure culture *Klebsiella* sp



Plate 8 posted mixed culture of *Bacillus* sp

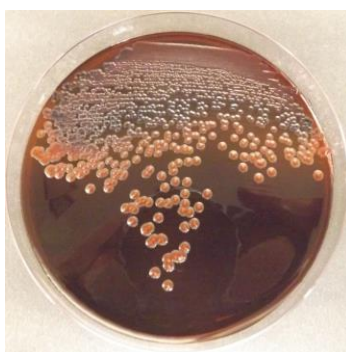


Plate 9 posted pure culture *Pseudomonas* Sp.

- Discontinuous swarming produces concentric circles around the point of inoculation.

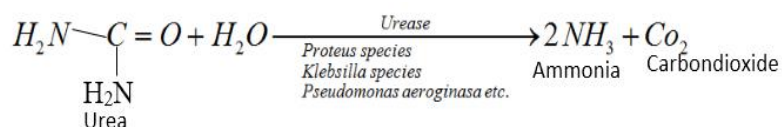


Plate 10 posted the swarming nature *Proteus* Sp



Plate 11 posted once again the swamping nature proteus Sp

All isolates were mostly gram-positive, rod-shaped, motile, catalase and oxidase positive as shown in Table 4. There were noticeable morphological differences among the isolated urease-producing bacteria. The diversity of the bacterial community in a given habitat could be limited due to alkalinity condition. Of all the ureolytic organisms isolates some *Bacillus* sp were capable of growing in these conditions (pH 7.0-7.8) while others in not less than 6.8-7.0 (Achal *et al.*, 2010). The close morphology of bacterial isolates were observed among the isolates and it might be as a result of the dominance species which might occur during enrichment culturing period since *Bacillus* species are usually selected by the isolation and cultivation methods (Stocks-Fischer *et al.*, 1999). The screening for urease producing bacteria was conducted using modified urea agar base medium in test tubes. The colour changes from pale yellow to pink-red indicated positive urease activity. All the six bacteria isolated from the tannery sludge waste were , selected based on the ability of the isolates to completely turn the urea agar base medium pink ; in comparison with other isolated urease producing bacteria which were previously studied for organic base bio-fertilizer and bio-seed bio-fertilizer((Burbank *et al.*, 2012). When urea is hydrolysed by the bacteria, ammonia is released and becomes accumulated in the medium which increases the pH of the environment making it alkaline (Hammad *et al.*, 2013).



Bacteria have developed controlled system to differentiate and cope up with harmful metal ions at minimal concentration. Assessing the effect of metal salts on ureolytic strains showed that all the strains were sensitive to Mercury Chloride (HgCl) Chromium Sulphate (CrSO₄) and Copper Sulphate (CuSO₄), while ZnSO₄ and CaSO₄ on uroelytic strain bacteria growth show a bit of tolerance growth level of ZnSO₄ and CaSO₄ up to optimal concentration of 2 % in table 5. The study reveals that most metals ion whenever they are opportune to enter bacterial cells they normally produced physiological and toxic effect to bacteria at minimal concentration, but a high concentration level the reverse is always the case(deadly to microorganisms) (Datta *et al.*, 2015)

Generally speaking the synergy symbiotic nitrogen fixation relationship between the root and the ureolytic bacteria are so restricted by so many factors. In view of the current investigation in Table 6 regarding to pH and temperature in-situ, temperature and pH are the major physical parameter that determine the growth and activities of microorganisms in a given habitat. With respect to the table 6 the control modified medium conditioned at 3.0, 4.0, 6.0, and 7.0 reveals that the ureolytic strains of bacteria thrived at pH 6 to 7. According to (Deora and Sinhel, 2010) have proposed that slight variation in the pH of the medium might have an enormous effect on the growth of ureolytic strains of bacteria. ureolytic strains of bacteria isolates were observed to be more sensitive to low pH than their host and this affect the consortium establishment of the symbiosis, limiting the survival and persistence of the ureolytic bacteria strain (Zahran, 1999). On the other hand Temperature is also one of the major factors affecting ureolytic strain of bacteria growth, survival in the soil and the symbiotic process its self (Niste *et al.*, 2013). High soil temperatures in the tropic have always been a major constrains of ureolytic bacteria consortium for BNF in cereal crops. High temperature may affect symbiotic relationships, nitrogen content, formation of roots and binding of ureolytic strain of bacteria to root hairs, development and function and dry matter production (Boboye *et al.*, 2011). Low temperature affect ureolytic bacteria as the process initiation is completely inhibited under low (Sadolosky, 2005). High temperature also alters the pattern of cell surfaces components (EPS and LPS) secreted by ureolytic strain of bacteria which in turn negatively affect the N₂ fixation (Yadav *et al.*, 2010).

Some agricultural waste or organic waste matter nowadays which have been perpetually looked upon with little or no values to Nigerian, if harness quite well they could be nutritionally used as food in Nigeria. Nigeria is bless with abundant natural waste resources but the technological knowhow for converting waste to wealth concept is totally and inadequately lacking, therefore the mass waste that is always being dispose in bushes have turned out to be a problematic(nuisance) to the society. In China and India nothing is gone wasted. Thus if Organic waste are properly harness quite well they could be use as food for the microbial single cell production (SCP) i.e. for protein enrichment. They could also improve flavor, protect food against deterioration and enhance nutritive values. Among the most important practical achievements of the use of localized available organic waste to modified some bacteriological growth medium so as to reduce the cost of importation of conventional medium in our current investigation, regarding organic base bio-fertilizer. Table 7 has shown the results of proximate composition nutritional values of some processed organic matter in readiness for carrier base organic base bio-fertilizer formulation whether in unfermented form or fermented form. The composition of enrich processed organic waste like those of Bean pod powder, rice bran waste powder, Banana peel waste powder, soya bean pod waste powder blood paste/blood meal waste powder, egg shell powder etc respectively. For various processed of the says samples where the moisture content, ash content, crude proteins, crude lipids, carbohydrate, nitrogen, phosphate etc respectively were vigorously and thoroughly analyzed for a way forward. The pH values ranged between 6.7 – 7.6 the study is in line with the report of (Deora and Singal, 2010) which they have early pro pose in their findings, that any slight variation in pH of medium might have an enormous effect on the growth of Ureolytic bacterial strains. Ureolytic bacteria were observed to be more sensitive to low pH than the host and this affect the consortium establishment of the symbiosis and thereby limiting the survival and persistence of the ureolytic bacteria (Zahran 1999). Moisture content of the rice bran waste powder had the highest values with 5.53% followed by soya bean pod waste powder. These findings were in agreement with the report of Audu and Aremu (2011) that the moisture content of a red kidney bean increases with (33.3%). Generally speaking the different processing technique employed to this study increasing moisture content in this order (0.22%<, 1.35%<, 1.51% <3.24%, 5.53% respectively. The increase in moisture content might be due to the water absorption by the fibers and other natural chemical composition during heat treatment While too much moisture content will have not be susceptible for microbial attack (Ejigiu *et al.*, 2005). Ash content of the Soya bean meal had the highest with 34.60% followed by Banana peel waste powder with 31.40% and that was found in blood meal with 23.6%. The Ash content in this very current investigation was variably higher than those values obtained by (Abitogun *et al.*, 2010). The difference in the values for Ash could be clearly explained by maturity stage and volatilization. Crude proteins is needed for normal body growth, repairs and maintenance for animals, plants and microorganisms. A relatively high amount of protein is therefore required for supplementation. In this study there was a significance ($P<0.05$) decrease in protein value of Banana peel waste powder with 1.24%. The highest crude proteins were found in chocolate blood paste with 39.4% while the least was found in Banana peel waste powder with (1.24%). These values compared favourable with the crude proteins values reported for *Zanthoxylum zanthoxyloids* (Nnamani *et al.*, 2009). Carbohydrate level in the soya bean meal was 44.6% which was significantly $P<0.05$ higher than those of rice bran (42.31%), Bean pod waste (38.4%) and others were found to be least. It could also be that a decrease in protein level of rice bran may have resulted in increase in carbohydrate. Further investigation share more light regarding to the high carbohydrate level which also indicated a good source of immediate energy for normal cell function in both unicellular and multicellular organisms. Crude fiber is part of food that is not easily digested by animals, microorganisms and humans. In animals as the normal functioning of the intestinal tract depends upon the presence of adequate fiber. It increase stool bulk and decreases the time that waste material spend in the gastrointestinal tract, it also help in the maintenance of human health and has been known to reduce cholesterol level of the body (Bellow *et al.*, 2008). Low fiber diets has been associated with heart disease, cancer of the colon and rectum Varicose veins, Phlebitis, Obesity, appendicitis, diabetes and even constipation. Crude Lipid content obtain for various organic waste matter was found in the decreasing by this order 7.45%> 6.23%>, 5.23% etc respectively Crude lipid provides very good source of energy and aids in transport of fat soluble vitamins, insulates and protect internal tissues found in both unicellular and multicellular organisms (Pamela *et al.*, 2005). More so it is idly to add lipid fat to most of our diets, because many body functions depend on lipids. Minerals are considered to be essential in human nutritional and generally, minerals from plants sources are less bio-available than those from animals source (Lopeo *et al.*, 2002). The results obtain from Table 7 of varieties of organic waste processed in readiness for organic bio-fertilizer productions. Blood meal had the highest nitrogen with 13.5%, followed by Soya bean meal with 4.02% the least among all the results was from banana peel waste powder with 0.59%.

The result in table 7 has Shown that the Nitrogen content in blood meal had the highest with 13.50% followed by soya bean meal with 4.02% the least of all the was 0.59% from banana waste peel powder. The findings have fallen to agreed with that of (Adegunwa *et al.*, 2012). Nitrogen is a major requirement for high yield of cereal crops and Nitrogen fertilization are often essential on soil of low organic matter as could be visualized or observed in table 7. Crops response to Nitrogen fertilizer is influence by factors such as Nitrogen fertilizer management soil types, crop sequence and supply of residual and mineralized nitrogen (Mangel

and Kirkby, 1987). Nitrogen presence in plant leads to the stimulation of leaf growth and stem; it's also gave a green coloration of the leaves. Deficiencies; lack of Nitrogen is often indicated by yellowish colour of the leave and short growth of the stalk. Posted in plate 12, 13 and 14



Plate 12 posted maize growth using urease biofertilizer (bioseed)



Plate 13 posted maize growth planted on the same time alongside without fertilizer



Plate 14 posted maize growth with bio-seed at once without application bio-fertilizer again

Phosphorous result was obtained during and after processed analysis of nutritional values of organic waste and other chemical analysis. Banana peel waste had the highest value with 4.78% followed by blood meal with 3.56% the least of all was observed in Bean pod waste with 0.51% as well as Potassium result was obtained during and after processed analysis of nutritional values of organic waste and other chemical analysis. Banana peel waste had the highest value with 14.70% followed by blood meal powder with 5.67%

and the least of all was found on rice bran 0.78%. The results of these findings have fallen in line with that of (Gopalan et al., 2000). Minerals are quantitatively minor compounds essential for the life because they contribute to multiple and different vital functions in the organism, like bone structure, homeostasis, muscular contraction, metabolism via the enzymatic systems, etc

The results in table 8 showing the pH differences for urea chemical fertilizer A, B and C with A 7.1 (a bit neutral) while for the bio-fertilizer/ bio-seed where their mean average was 7.25(a bit Neutral). Varying in pH values of the both the chemical fertilizers (A and B/C) as well as for bio-fertilizer(C) in the soil might altered the rate of biological reaction and the survival of various microorganisms at this particular range of pH 6.8 and 7.0. Thus the organisms might absolutely and sincerely maintain their level of integrity in terms of improving the soil fertility for their survival as well as the life of plants and animals (Bonnate *et al.*, 2008). The varying in the pH of both chemical fertilizer (A and B/C) could also attributed to the chemical content. Moisture content for both chemical fertilizer (A and B/C) differed from one another was base on the formulation composition and ranged between 2.40 % – 4.45% . Varying in the moisture content of each respected fertilizer could also be as a result of composting decrease due to incubation period because . Thus the inoculation of the bio-fertilizer with EM increased the temperature and decrease the moisture content bio-fertilizer and the reverse is more or less the to the chemical fertilizer. The same phenomenal has also been notified in open field composting (Pai et al 2003). There was also varying of ash content that was notice within the axes of chemical fertilizer which had fallen between the range of 7.20%- 24.52 % as well as for the bio-fertilizer/bio-seed fertilizer which were observed to be 24.51/24.52. This could be attributably due to the maturity of composting, the ash content significantly increase during preparation. Since the organic materials were decomposed to form the metabolic gases (Chang and Yang, 2009).

Crude proteins are needed for normal body growth, repairs and maintenance for animals, plants and microorganisms. A relatively high amount of protein is therefore required for supplementation. In this study there was a significance ($P < 0.05$) decrease in protein value urea Chemical fertilizer 18.56%. The highest crude protein was found in bio-seed/ bio-fertilizer with 23.23%/ 23.10% respectively. These values compared favorable with the crude proteins values reported for *Zanthoxylum zanthoxyloids* (Nnamani *et al.*, 2009).

As shown in table 8 the results obtained from sample A, B, C content A(1.72) of fat content while B and C(4.41% and 4.485) of fat content respectively. The finding is agreement with those of (Nnamani *et al.*, 2009). Crude lipid provides a very good source of energy and lipid to plant, microorganisms and animals; it aids in the transport of vitamin, insulates and protects internal tissue and contributed immensely to transportation of cells processes.

Carbohydrate level in urea chemical fertilizer was 23.40% which was significantly $P < 0.05$ less than those of B with (44.60%) and C with (44.61%) respectively. It could also be that a decrease in protein level of of A, B, C may have resulted in increase in carbohydrate. Further investigation share lighter regarding to the high carbohydrate level with also indicated a good source of immediate energy for normal cell function in both unicellular and multicellular organisms

In the process of time there were gradual increase of crude fibre starting from both chemical fertilizer A B/C at the ranged of 2.45% to 7.82% and the highest were observed from bio-fertilizer/bio-seed with 7.65 % and 7.82%. The results of crude fiber were in agreement with the findings of (Abitogun et al., 2010) and (Harbrne, 1973) respectively. On the contrary (Singh and Yadav, 1978) reported fewer values and it ranged between 9.10%- 13.07%, while (Abitogun et al., 2010) was reported 12.7 % CF; this could also due to changes in the dry matter of both chemical fertilizer and Bio-fertilizer/bio-seed.

Urease organic base bio-fertilizer has the highest nitrogen percentage with 16.34% followed by the bio-seed organic base bio-fertilizer with 16.32% and the least was found in urea chemical fertilizer (13, 12%) the study agreed with the report of (Bakonyi *et al.*, 2013). Nitrogen is the most important fertilizer.... . Plants respond quickly to application of nitrogen. The element encourages above ground vegetation growth and gives a deep green colour to the leaves. Plant root take up nitrogen in the form of NO_3^- and NH_4^+ (Bakonyi *et al.*, 2013) It is the most important major nutrients required by plant for proper growth and development; it also a part of all living cells. It is a necessary part of all proteins, enzymes and metabolic processes involved in the synthesis and transfer of energy (68). Nitrogen cycle plays an important role in soil system and influenced by biological processes. Its required for the growth of plant and is a constituent of chlorophyll, plant proteins and nucleic acid (Choudhury, and Kennedy, 2004).

The results obtain for Phosphate analysis of both chemical fertilizer urea and urease organic bio-fertilizer is found to be in this increasing order $A < 5.15\%$, $C < 12.60\%$, $B < 12.60\%$ respectively. The study is in accordance with the report of (Choudhury, and Kennedy, 2004). Phosphorus is part of every living cell in plant. It is one of the most important Macro-element essential for plant growth. Phosphorus is the most often limiting nutrient remains present in plant nucleic acid and act as energy storage. It helps in transfer of energy (Choudhury, and Kennedy, 2004). It is also an essential part of the process photosynthesis, involved in the formation of cell oils, sugar, starch etc (Aher *et al.*, 2015). Phosphorus is abundant in the fruit of plant and seeds and also plays an important role in plant processes. Similarly Wagh and Sayyed said that phosphorus is necessary for seed germination and essential for flowering and fruit formation. It deficiency sym toms are purple stem and leaves poor yield of fruit (Wash *et al.*, 2013), reported

that phosphorus is one of the key macronutrient required for plant growth and metabolism. The most of the activity of plant such as growth respiration and reproduction depends upon phosphorus level of the soil in which plant growth. Potassium result was as obtain in table 8 for both chemical urea fertilizer and bio-seed/ bio-fertilizer urease base were in this order B had the highest value of 13.56%, followed by C with 13.46% and the least was observed in A with 2.41%. These results are in agreement with the report of (Ifokwe, 1988). Potassium is ascribed by plants in form of potassium ions which is insoluble in water. It is added to the soil by fertilizer decaying organic matter and wood ash. According to (Ifokwe, 1988), Potassium deficiency upsets plant metabolism, inhibits the activities of some enzymes and disrupts carbohydrate supply reduces, the viability of seeds make plant more prone to disease and when harvested, they become difficult to preserves in marketable conditions. Plate 15, 16 and 17



Plate 15 posted urease base organic bio-fertilizer



Plate 16 posted urea chemical fertilizer



Plate 17 posted Modify organic base bio-seed fertilizer on display.

Table 1 Micro climatic data at the consortium

PARAMETERS	
Temperature ($^{\circ}\text{C}$)	34 ± 4
Average Annual Rainfall (mm)	191 ± 9
Relative humidity	51 ± 5
Light Intensity (cd)	3000 and above

(Source: NARICT, 2019)

Table 2 Properties of the soils at consortium of both contaminated and control landfill

	CONTROL LANDFILL(NARICT)	CONTAMINATED LANDFILL(YANKUSA)
Structure	Upper loss Lower Compacted	Fairly loss/gumming Lower compact
Texture	Upper – fine Lower particulate	Upper fine/mixed with coloured chemical waste debris/blue/black and greenish Lower particulate
Composition	Upper sand/humus Lower sandy	Mixture of sandy humus Cr ion and tanning debris Lower Laterite and plutonic particulates
Moisture	Upper dry surface	Upper dry some time wet surface
Stability	Periodical addition due to natural erosion	Periodical addition of chemical waste discharge and erosion

Table 3 Morphological study of ureolytic bacteria strains

METHODS	PROPERTIES	BACTERIALS ISOLATES
Gram staining	Gram Reaction Cell shape Size(Um) Arrangement	+ve Rods 4um Occurring single as well as in chain
Capsule staining	Gram reaction Cell shape Size(Um) Arrangement	+ve Rods 4um Occurring in single
Spore staining	Gram reaction Cell shape Size (um) Cell arrangement	None - None None
Light Microscopy	Motile	Ve+ as well as swamping

Table 4 biochemical characterization of ureolytic strains of bacteria isolates

Character	<i>Bacillus</i> sp	<i>Pseudomonas</i> sp	<i>Proteus mirabilis</i>	<i>Klebsiella</i> sp	<i>Enterobacter</i> sp
Intra cellular enzyme					
Aerobid facuta	(A) A/FA	A	F/A	F/A	F.A
Catalose activities	+	+	+	+	+
Oxidase activity	+	+	+	+	+
Indole production	ND	ND	+/-	+/-	-
Citrateutilization	-	-	+/-	+/-	+
Urease activities	ND	ND	+	+	+/-
H2s. production	ND	ND	+	-	-
Methyl red	ND	ND	+	-	+
Vogesproskau	ND	ND	+/-	+/-	-
Lactose	+	-	-	+	+

Glucose	+	+/-	+	+	+/-
Sucrose	+	+	+/-	+/-	+/-
Mannitol	+	+	+/-	+/-	+/-

Extra cellular enzyme

Starch hydrolysis	+	+	+	+	+
Lipid hydrolysis	+/-	ND	ND	+/-	+/-
Gelatine hydrolysis	+	+	+/-	+	+/-

Key=characters

Table 5 Effect of metal ions on growth of urease strains

METAL SALT	UREASE STRAINS	% GROWTH CONCENTRATION			
		0.0	0.1	1	2
Hgcl ₂	"	+	-	-	-
CrSO ₄	"	+	-	-	-
ZnSO ₄	"	+	+	+	+
CaSO ₄	"	+	+	+	+

Table 6 Effect of ph and temperature on tolerance level of ureolytic bacteria strains at 0.1 % to 1% znso₄/caso₄ concentration in modified rhobium growth agar (MRA) for which different general score positive (+) negative (-) ND = Not done

Isolates	Colony growth mm at 0.1% concentration (maximum)							
	pH					Temperature °C		
	3.0	4.0	5.0	6.0	7.0	24	27—32	35—45
<i>Bacillus sp</i>	--	-	-	-	+++	-	++++	++
<i>Proteus mirabilis</i>	--	-	-	-	+++	-	+++	++
<i>Pseudomonas sp</i>	--	-	-	+	++++	-	+++	+
<i>Klebsiella sp</i>	--	+	+	--	++++	--	+++	+++
<i>Enterobacter sp</i>	-	-	-	-	+++	-	++++	+

KEY + + + + Abundant growth + + + Moderate growth + + Scarce + Less - None

Table 7 Processed nutritional value of organic waste and other chemical analysis for biofertilizer production

MATERIALS	PERCENTAGE (%)							MACRONUTRIENTS (%)		
	pH	Moist	Ash	Crude Protein	crude lipid	Carbo Hydrate	Crude fibre	N	P	K
Bean pod waste powder	7.3	1.51	30.21	5.70	3.80	38.4	8.81	2.56	0.51	1.24
Rice bran waste powder	6.7	5.53	29.34	6.76	5.23	42.31	11.3	1.06	1.53	0.78
Banana waste peel powder	6.8	1.35	31.40	1.24	2.90	34.61	6.20	0.59	4.78	14.7
Soya bean pod waste powder	7.2	0.22	27.80	7.58	3.46	36.59	7.20	1.21	1.45	1.05
Soya meal	7.6	5.41	34.60	29.4	6.23	44.6	5.62	4.02	2.25	2.45

Blood paste/blood meal powder	7.1	3.24	23.6	39.40	7.45	24.60	3.12	13.50	3.56	5.67
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Table 8 Macro and micro nutrient and other chemical analysis for biofertilizer A B C

Biof Form	PERCENTAGE (%)							MACRONUTRIENTS (%)		
	pH	Moist	Ash	Crude Protein	Crude lipid	Carbo hydrate	Crude fiber	N	P	K
A	7.1	2.40	7.20	18.56	1.72	23.4	2.45	13.12	5.15	2.41
B	7.2	4.51	24.51	23.10	4.41	44.60	7.65	16.34	12.60	13.56
C	7.3	4.45	24.52	23.23	4.48	44.61	7.82	16.32	12.37	13.46

Keyword: A= chemical Urea, B= Urease base organic bio fertilizer, C=Bio-seed organic base bio-fertilizer

4. CONCLUSION AND RECOMMENDATIONS

It was concluded that most organic waste contain an appreciable amount of macro-nutrient/ microelements, especially protein and carbohydrate which is advised to be used as bio-fertilizer in our yearly farm land. This will reduce the risk of nutrients deficiency in plants and microorganisms. Moreover, locally available organic waste should be modified so as a tangible bio-fertilizer would be readily made available and which is invariably recommended for improving the soil fertility in organic farm, it stimulate plant growth, it help in building soil micro-flora and thereby soil health as well as providing resistance against drought and soil- borne disease. In current agriculture practices, chemical fertilizers have reduced the fertility of soil, making it unsuited for raising crop plants. Additionally, the excessive use of these inputs has also led to severe health and environmental hazards such as soil erosion, water contamination, pesticide poisoning, falling ground water table, water logging and depletion of biodiversity. Bio-fertilizers spontaneously activate the microorganisms found in the soil.

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

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Data and materials availability:

All data associated with this study are present in the paper.

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